

150 CF epithelial cells are primed for apoptosis as a result of increased Fas

S. Weldon¹, Q. Chen¹, C. Wohlford-Lenane², J.A. Bartlett², J.S. Elborn¹, P.B. McCray Jr.², C.C. Taggart¹. ¹Queen's University Belfast, Centre for Infection and Immunity, Belfast, United Kingdom; ²University of Iowa, Department of Paediatrics, Iowa, United States

Apoptosis is a physiological process essential for homeostasis of epithelial organisation and function. CF lung disease is characterised by chronic infection and inflammation and previous work suggests that apoptosis is dysfunctional in the CF airways with conflicting results. In addition, controversy exists regarding how CFTR misfolding contributes to apoptosis. In this study, we evaluated the relationship between CFTR mutation and apoptosis in $\Delta F508$ -CFTR CF airway epithelial cells. Basal activity of the executioner caspase, caspase-3, was significantly increased in CF tracheal and bronchial epithelial cell lines and primary bronchial epithelial cells compared to non-CF controls. In addition, activity of the upstream initiator caspase, caspase-8, was significantly increased in CF epithelial cells compared to controls, suggesting involvement of extrinsic apoptosis signalling, which is mediated by the activation of death receptors, such as Fas (CD95). Increased levels of Fas were observed in CF epithelial cells, and neutralization of Fas significantly inhibited caspase-3 activity in CF epithelial cells compared to untreated cells. Furthermore, activation of Fas significantly increased caspase-3 activity and apoptosis in CF epithelial cells compared to control cells. Overall, these results suggest that CF airway epithelial cells are more sensitive to apoptosis via increased levels of Fas and subsequent activation of the Fas death receptor pathway.

151 Investigation of MicroRNA regulation of interleukin-8 production in bronchial epithelial cells

K. Gaughan¹, T. Hassan¹, N.G. McElvaney¹, C. Greene¹. ¹Royal College of Surgeons Ireland, Respiratory Research Division, Dublin, Ireland

Objectives: The major cause of morbidity and mortality in Cystic Fibrosis is gradual deterioration of pulmonary function. This has been attributed to an excessive inflammatory response to chronic infection. The release of Interleukin-8 (IL-8), from bronchial epithelial cells in particular, and the subsequent influx of neutrophils into the lungs play a large part in this process. Lowering IL-8 production, by modulating microRNAs (miRNAs), could have therapeutic benefit. miRNAs are non-coding RNAs that regulate gene transcription by degrading or preventing translation of mRNA. Here we investigate miRNAs that regulate IL-8 gene expression.

Methods and Results: A search for miRNAs that target IL-8 was performed in silico (TargetScan, microRNA, PITA and Microcosm Targets) and a shortlist of possible miRNA candidates was created (n=15). In parallel IL-8 mRNA-miRNA complexes were isolated from human 16HBE140⁺ bronchial epithelial cells using custom designed biotinylated DNA oligonucleotides complementary to exposed sequences in the IL-8 mRNA. IL-8 mRNA, but not other transcripts, was isolated with its cognate miRNAs bound.

Conclusions: Profiling and validation of these IL-8 targeting miRNAs will identify miRNAs that regulate IL-8 and represent new therapeutic targets for CF.

152 Estrogen-regulated MicroRNAs control the expression of secretory leukoprotease inhibitor in monocytes

P. McKiernan¹, S.H. Chotirmall¹, S.A. Cryan², N.G. McElvaney¹, C. Greene¹. ¹Royal College of Surgeons in Ireland, Respiratory Medicine, Dublin, Ireland; ²Royal College of Surgeons in Ireland, School of Pharmacy, Dublin, Ireland

Objectives: The levels of the circulating estrogen 17 β -estradiol (E2) have been viewed as an influencing factor on the progression of many lung diseases including the gender dichotomy that exists in Cystic Fibrosis (CF). We hypothesize that E2 modulates the inflammatory response of circulating innate immune cells through microRNA (miR) based modulation of Secretory Leucoprotease Inhibitor (SLPI), a multifunctional antiprotease.

Methods: Monocytic cells [THP-1s, U937s and peripheral blood monocytes (PBMCs)] were treated with 10 nM E2 or ethanol vehicle control for various times up to 48 hours. miRs that were differentially expressed in THP-1s were identified using Taqman Low Density Array. U937 cells were transfected with premiRs (miR mimics) or anti-miRs for 48 hours. SLPI mRNA and protein levels were quantified by q-RT-PCR and ELISA, respectively.

Conclusions: SLPI expression is downregulated in response to E2 in monocytic cells (THP-1, U937 and male PBMCs). Of the 768 miRNAs profiled two of those miRNAs that were upregulated by E2; miR19a and miR19b; are predicted by *in silico* analysis to directly target SLPI mRNA. Transfection of U937s with premiRs 19a or 19b reduced SLPI expression at both mRNA and protein levels. This was abrogated using anti-miRs against the same miRs. The data show that E2 decreases expression of SLPI in monocytic cells, likely via changes in miRNA expression. Modulation of the miRNAs involved in E2-dependent regulation of SLPI expression offers a new therapeutic approach in the treatment of inflammatory lung diseases such as CF.

153 Regulation of corticosteroid binding globulin (CBG) in the inflammatory context of cystic fibrosis

J. Taytard¹, C. Rebeyrol¹, J. de Baaij¹, D. Debray¹, O. Tabary¹, L. Guillot¹, H. Corvol^{1,2}, A. Clement^{1,2}, N. Chignard¹, P. le Rouzic¹. ¹UMR S938/Univ Paris 06, Paris, France; ²AP-HP, Hôpital Trousseau, Pediatric Pulmonary Department, Paris, France

Background: Cystic Fibrosis (CF) is characterised by chronic lung inflammation. In CF, glucocorticoids (GC) are a widely used therapeutic tool. However, their efficiency, and the benefit/risk ratio are still discussed. In plasma, 90% of GC is bound to the chaperone protein corticosteroid-binding globulin (CBG) produced by the liver. Recent works enlightened the fact that, more than a simple carrier protein, CBG could also address GC specifically to the inflammation site, thereby modulating the response to GC in an inflammatory context.

Aims: Study the expression and regulation of CBG in the inflammatory context of CF.

Methods: Hepatic expression: hepatocarcinoma derived cell-lines and biopsies from healthy donors, cirrhotic CF and non CF patients: measure of transcripts and protein levels of CBG.

Plasmatic levels: Blood samples from healthy donors, cirrhotic non CF and CF patients: measure of CBG.

Lung expression: Bronchial epithelial cell lines; regulation of CBG expression.

Results: We observe an increase in CBG transcript and protein in the liver of CF patients and in the hepatic and lung cell lines under pro-inflammatory challenge. We show that GC have no effects on CBG expression in hepatic cell lines, but increases CBG transcripts in the lung cell lines. Surprisingly, we observe no CBG up-regulation in the blood of CF patients.

Discussion: Comparative results from hepatic and lung cell lines enlighten a cell-specific regulation of CBG. Moreover, the transcript CBG up-regulation observed in CF patients is not correlated to CBG plasma levels. We hypothesise that such lack of increase in plasmatic CBG could participate to chronic lung inflammation in CF.